Like other integrins, αIIbβ3 can signal bi-directionally through interaction of its ECD with extracellular ligands (''outside-in'' signaling) or its CT with intracellular proteins (''inside-out'' signaling). Notwithstanding the recent developments in structural biology of integrins, questions remain about the molecular determinants that are responsible for protein activation and signaling. To obtain rigorous mechanistic insights into αIIbβ3 ''inside-out'' signaling at an unprecedented level of molecular detail, we carried out microsecond-scale all-atom molecular dynamics (MD) simulations of various experimentally-based three-dimensional models of the TM and CT regions of αIIbβ3 in an explicit 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocoline bilayer, and in the presence or absence of the F2-F3 subdomains of the intracellular activator talin-1.

These simulations broaden our current understanding of the mechanism of αIIbβ3 activation by talin-1. Specifically, we observe: a) different interactions between the αIIb and β3 CTs with or without the F2-F3 subdomains of talin-1; b) a modulatory effect of F2-F3 on the αIIbβ3 TM and CT structures, c) specific electrostatic interactions between talin-1 F2-F3 and the phospholipid polar groups; and d) flexibility of the linker region between the F3 and F2 resulting in their re-orientation with respect to the corresponding crystal structure. Notably, these simulations reveal novel stable interactions between residues of the αIIb and β3 CTs (E1005-K725, E1006-K729, and E1008-R736, respectively), the αIIb CT and the talin-1 F3 (E1001-K316 and D1004-K364, respectively), as well as the β3 CT and the talin-1 F2-F3 (E726-K316, E726-Q381, T753-N355, F754-Y373, and R760-E293, respectively), the mutation of which may be worthy of experimental testing based on their expected interference with αIIbβ3 activation.

2027-Pos Board B797

Molecular Dynamics Comparison of Electroporation in Water-Vacuum-Water and Lipid Bilayer System

Ming C. Ho[1], Zachary A. Levine[1,2], P. Thomas Vernier[2,3].
[1]Department of Physics and Astronomy, Dornsife College of Letter, Arts, and Sciences, University of Southern California, Los Angeles, CA, USA, [2]MOSIS, Information Sciences Institute, University of Southern California, Marina Del Rey, CA, USA, [3]Ming Hsieh Department of Electrical Engineering. Viterbi School of Engineering, University of Southern California, Los Angeles, CA, USA.

A recent study showed that water forms cone-like structures at a vacuum or air interface under a sufficiently high electric field [1]. This protruding water formation may be a key step in the initiation of electroporation of lipid bilayers and cell membranes. In molecular dynamics (MD) simulations of water-vacuum-water (WVW) systems, we observe the intrusion of water into the vacuum region, similar to the pore initiation stage of electropore formation in phospholipid bilayer systems [2]. Pore initialization time in WVW systems is found to decay exponentially with applied electric field, consistent with simulations of lipid bilayers [2]. Pore initialization time in WVW systems also increases exponentially with increasing vacuum gap size. We analyze pore creation, pore annihilation, and pore stabilization. By comparing the evolution of the pore radius in both systems, we can begin to quantify how water and phospholipids each contribute to the formation and stability of lipid nanopores.

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2028-Pos Board B798

Calculating the Free Energy of Antimicrobial Peptide (HHC-10) Aggregation in the Bulk

Shaqa Vafaei[1], Mostafa Nategholeslam[1], Matthew Nichols[2], Miljan Kuljanin[2], Masoud Jelokhani-Niaraki[2], Bruno Tomberli[3], Chris Gray[1].
[1]University of Guelph, Guelph, ON, Canada, [2]Wilfrid Laurier University, Waterloo, ON, Canada, [3]Brandon University, Brandon, MB, Canada.

The increasing demand for antibiotics has contributed to the investigation of possible novel antibiotics by many researchers. For this purpose, experimental and theoretical studies have been carried out to draw scientists' attention to antimicrobial peptides and their interaction with the surface of bacterial membranes. Their ability to disrupt the functioning of bacterial membranes has been probed from different perspectives. The best possible choice of antimicrobial peptides are those which do not harm plant or animals' membranes but which disrupt bacterial membranes. It has been found that some cationic antimicrobial peptides (CAPs) satisfy these requirements. CAPs interacting with the outer membrane of gram-negative bacteria and the membrane of gram-positive bacteria has been studied recently.

We conduct a MD simulation study of peptide-peptide interactions in the physiological solutions and investigate the mechanism of CAPs aggregation, since aggregation of the peptides usually precedes formation of a pore in the membrane. Different algorithms will be applied to calculate the potential mean force of the aggregation process of peptides to select the most efficient one. Also, we have run CD spectroscopy and Calorimetry experiments to predict the structure of the peptide and measure the peptide-peptide binding enthalpy, and we compare the results with our simulation data.The particular CAP studied is HHC-10, a peptide designed by the Hancock group, which has 9 amino acid residues and charge +4.

2029-Pos Board B799

Free Energy Calculations of the Transferring of a Cyclic Arginine 9 Peptide across a Lipid Bilayer Suggest a Water-Pore Assisted Mechanism

Kun Huang, Angel E. Garcia.
Rensselaer Polytechnic Institute, Troy, NY, USA.

Molecular details of translocation arginine rich cell penetrating peptides through lipid bilayers are still under debate. Any mechanism has to answer the central question that how a highly hydrophilic peptide is able to cross the hydrophobic core of lipid membrane. A possible mechanism involves the formation of a local water defect in the membrane such that the hydrophilic residues of the peptide are solvated throughout the translocating process. In this work, we calculate the free energy of forming a water pore, the translocation of the peptide with the pore formed, and translocation of the peptide without a water pore. Free energies are calculated along order parameters using umbrella sampling. The calculations shows that free energy of transferring the peptide into the bilayer through a path involving a water defect is lower than the path without a water defect. The free energy of forming a pore is around 100 KJ/mol. We will discuss the unsuitability of different pathways to describe the kinetics of peptide translocation. We expect these results could extend to arginine rich peptides, such as antimicrobial and cell-penetrating peptides, in general.

2030-Pos Board B800

Palmitate Effect on the Trasmembrane Domain of IRE1α Protein

Francesca Stanzione[1], Amadeu K. Sum[1], Hyunju Cho[2], Cristina Chan[2].
[1]Colorado School of Mines, Golden, CO, USA, [2]Michigan State University, East Lansing, MI, USA.

The Inositol-requiring kinase 1α protein (IRE1α) is a transmembrane protein kinase essential for the endoplasmic reticulum (ER) unfolded protein response that promotes cell survival by reducing the levels of misfolded protein. IRE1α consists of an N-terminal luminal domain, a single-pass transmembrane spanning segment and a cytosolic region which contains a kinase domain and an endoribonuclease (RNase) domain. Under conditions of ER stress, the transmembrane protein IRE1α oligomerizes to activate its cytoplasmic kinase and RNase domains. Recently, we showed that palmitate is involved in ER stress by increasing the kinase and RNase activities of IRE1α in human hepatocellular carcinoma (HepG2) cells. From our previous studies of palmitate in the membrane, palmitate aligns with the lipids. It is expected that palmitate will assume a similar conformation with the transmembrane (TM) domain, however, it is unclear whether palmitate binds directly to the TM domain or indirectly alter the structure of the bilayer surrounding the TM domain, thereby leading to enhanced association between the TM domains.

We combined experiments with molecular dynamic (MD) simulations to investigate the properties of the TM domain, the role of palmitate on IRE1α dimerization and to elucidate potential interactions between palmitate and TM domain. Since structural information of the TM domain of IRE1α is not available, we experimentally determined whether the predicted TM domain forms α-helical secondary structure in DOPC liposome using circular dichroism. In support of the MD simulations, we assessed whether palmitate enhances TM dimerization in DOPC micelles using Förster resonance-energy transfer. Further mutation study of the TM domain provided information of how palmitate is involved in modulating the cellular activity of IRE1α mediated by the TM domain. Finally, the computational and experimental studies provided new insight into the role of palmitate on IRE1α activity during ER stress.

2031-Pos Board B801

NMR Observable-Based Structure Refinement of Membrane Proteins in Explicit Membranes

Xi Cheng.
The University of Kansas, Lawrence, KS, USA.

Various NMR observables, such as chemical shift anisotropy (CSA) and dipolar coupling (DC) in solid-state NMR, and NOE-based distance and residual dipolar coupling (RDC) in solution NMR experiments, have been used to